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In mixed hematopoietic chimerism, the donor red cells win

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Allogeneic hematopoietic stem cell transplantation (HSCT) was first observed to correct the thalassemias and hemoglobinopathies over three decades ago.[1,2] Since then, over 3000 transplants for these disorders have been performed worldwide, and allogeneic HSCT currently remains the only proven curative therapy for these highly morbid and life-limiting diseases. In these settings, engraftment of donor-derived cells following HSCT serves to replace dysfunctional cells of the red cell lineage. For over a decade, it has been recognized that a subset of patients transplanted for these disorders intriguingly demonstrate stable and durable co-existence of nucleated donor cells with host cells and that this chimeric state is associated with transfusion-independence and the lack of continued clinical manifestations of their disease.[3,4] Now, in a study presented in this issue of the journal, Andreani *et al.*[5] definitively demonstrate that patients with long-lasting stable mixed hematopoietic chimerism (3 with thalassemia and 1 with sickle cell disease), including mixed chimerism of marrow erythroid progenitors, expressed a 2- to 5-fold enrichment of donor-derived mature erythrocytes in the peripheral blood.

It is important to put this study in the context of previous work. Data from the current study along with those of previous studies[3,6] are indeed similar. In the bone marrow, the percentage of donor myeloid cells correlates with that of erythroid cells, consistent with the current understanding of myelo- and erythropoiesis deriving from common myelo-erythroid progenitors.[7] Furthermore, in the peripheral blood the percentage of donor leukocytes is similar to that in the marrow; however, the percentage of donor red cells is much higher (Figure 1). This enrichment in donor peripheral red cells has also been observed in children with sickle cell disease after myeloablative transplants.[4] The observation of full replacement by donor-derived mature red blood cells occurring within these mixed chimeras provides an understanding of the dramatic functional improvements observed in these patients following allogeneic HSCT.

These observations support the long-held notion that erythroid precursors in the thalassemias and hemoglobinopathies are at a competitive disadvantage for generating mature red blood cells capable of exiting the marrow. The current data from Andreani *et al.*[5] are highly convincing as they were derived from patients exhibiting long-term (>3 years) stable chimerism who did not require red blood cell transfusions. The effects measured

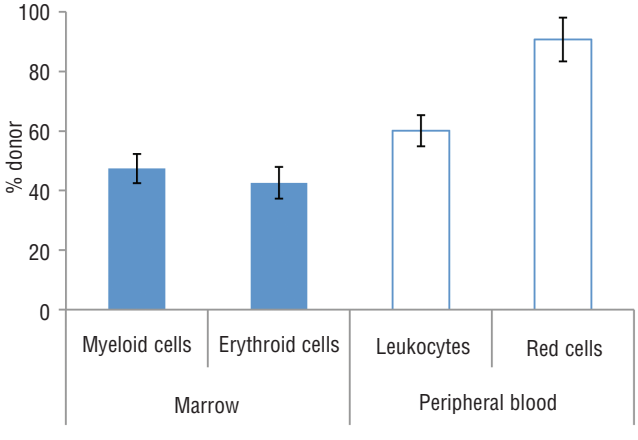

Figure 1. Comparison of marrow and peripheral blood donor chimerism. Results of two prior studies[3,18] along with those of the study by Andreani *et al.*[5] are summarized here. There were 16 samples of marrow myeloid cells, 13 of marrow erythroid cells, 31 of peripheral blood leukocytes, and 4 of peripheral blood red cells. The error bars refer to standard errors of the mean.

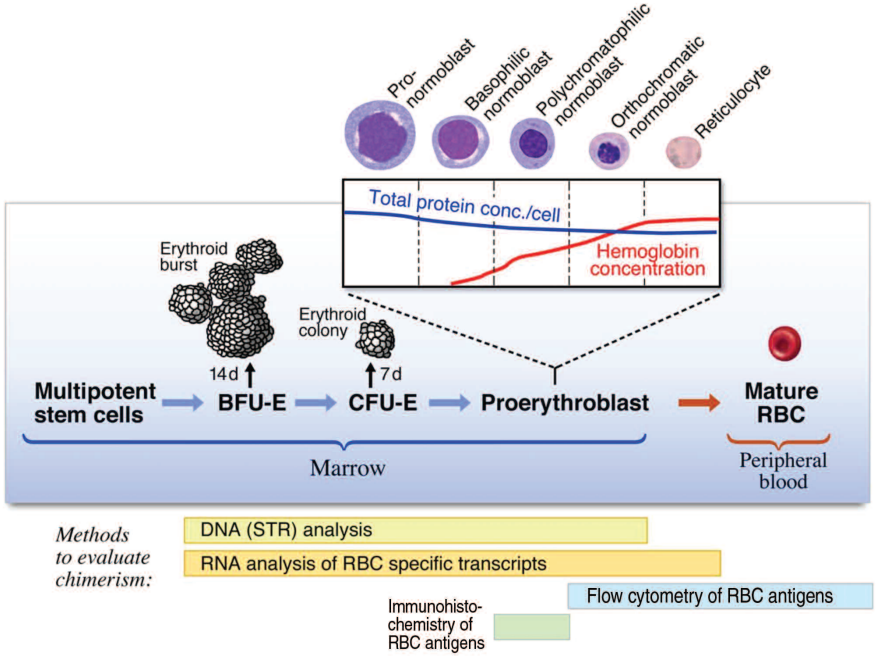

Figure 2. Erythroid maturation and methods to detect donor cells. Depending on the stage of erythroid differentiation and development, there are specific methods to assay the donor contribution.

are, therefore, highly unlikely to have been confounded by factors related to the transplant regimen. While Armistead *et al.*[8] utilized erythroid-lineage specific molecular markers to quantify donor *versus* recipient red blood cell transcripts in patients undergoing reduced-intensity and myeloablative HSCT regimens for thalassemia, Andreani *et al.*[5] adopted a more direct approach. As illustrated in Figure 2, they directly measured the donor contribution to red blood cell progenitors (BFU-E) by DNA (short tandem repeat) analyses and to mature erythrocytes by fluorocytometry to detect donor-specific red blood cell antigen expression. Their conclusions are well-supported by the literature, as ineffective erythropoiesis in the thalassemias and hemoglobinopathies has been described in both murine models[9] and patients.[10,11] Apoptosis is an important mechanism by which ineffective erythroblasts are cleared within the intramedullary space in both diseases, but particularly in thalassemia.[12,13] In sickle cell disease, expression of sickle hemoglobin in as early as basophilic normoblasts appears to lead to mechanical defects that in turn increase the cells' susceptibility to clearance and loss.[11,14-16] While the precise mechanisms by which these two disorders generate ineffective erythropoiesis differ, they share the common feature that ineffective erythropoiesis becomes apparent as cellular hemoglobin concentrations rise in the erythrocyte-committed precursors.

Accumulating evidence suggests that this mixed hematopoietic chimerism can be stable. One next logical question is: what is the lowest percentage of donor leukocytes that would provide nearly all donor red cells in the peripheral blood? As shown by the study by Andreani *et al.* (patient 41)[5] and earlier reports,[4,10] this percentage could be as low as 10 or 20%. This level is corroborated by the results of one patient in the gene therapy trial for thalassemia who received autologous hematopoietic stem cells genetically modified to express a modified β-globin and, at 1 year after therapy, became transfusion independent. By 2 years after therapy, the genetically modified cells accounted for 11% of peripheral blood leukocytes (18% granulocyte-monocytes), 11% of marrow erythroid colonies and 13% of marrow myeloid colonies.[17]

Collectively, these observations support mixed hematopoietic chimerism as a rational goal in non-malignant disorders. Another important question remains: what is necessary to attain this stable state of mixed hematopoietic chimerism? Furthermore, are T, B, or other lymphocyte subsets responsible for allowing this persistent and stable mixed chimerism? Lisini *et al.*[18] performed a preliminary analysis in 13 patients within the first year after transplant, finding that the average percentage of donor cells was 75% (range, 30-90%) for leukocytes; 52% (range, 10-80%) for CD4 cells, 44% (range, 10-80%) for CD8 cells and 90% (range, 60-95%) for CD19 cells. In their patients, there was high number of B cells, with generally 10-20% fewer donor T cells. However, Andreani *et al.*[6] found the opposite in their past series of six patients, with the percentage of donor CD19 cells being lower. The relatively small number of patients in both studies and the varying donor T- and B-cell contributions preclude any firm conclusions, and the mechanism of induction of stable mixed chimerism may not be related to cell number or percentage, but rather to cell type. Andreani *et al.*[3,6] also previously showed that, in their cohort of patients, the risk of graft loss was highest when the percentage of donor leukocytes was less than 75% in the first 2 months. However, this risk decreased dramatically when mixed hematopoietic chimerism persisted beyond 1 year. Others have identified heavy transfusion burden with inconsistent iron chelation and red cell alloimmunization as additional risk factors. It would be useful to determined whether there are other early (<3 months post-transplant) or late (>3 months) markers that could prospectively predict the risk of graft loss. The results published by Andreani *et al.* add significantly to our cur-

rent understanding of mixed hematopoietic chimerism in non-malignant disorders and provide the basis for several fertile areas of research. Furthermore, such clinical observations will be important in the design of future transplantation regimens for these devastating disorders.

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